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Graft copolymers of hydroxyethyl cellulose by a 'grafting to' method: ^{15}N labelling as a powerful characterisation tool in 'click' polymer chemistry

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We demonstrate how ^{15}N labelling can be used to probe the success of 'grafting to' processes, through the preparation of well-defined graft copolymers of hydroxyethyl cellulose by combining RAFT polymerisation and copper-catalysed azide-alkyne cycloaddition (CuAAC). Using synthesised alkyne-functionalised chain transfer agents, short-chain (DP=10) poly(N-vinylpyrrolidone) (PVP) and poly(N-isopropyl acrylamide) (PNIPAAM) were prepared in high conversion in a controlled manner ($Đ_M$ of ~1.4 and 1.2 respectively). Separately, partially ^{15}N-labelled N_3-HEC was synthesised and characterised using solid state ^{13}C, ^{15}N CP-MAS NMR and FTIR spectroscopies. Alkyne-terminated PVP and PNIPAAM were grafted at different graft densities onto partially ^{15}N-labelled N_3-HEC using the click reaction. The hybrid HEC-g-polymer materials were fully characterised using solid state ^{13}C and ^{15}N CP-MAS NMR and FTIR spectroscopies. While ^{13}C and FTIR spectroscopies gave indirect or weak evidence of CuAAC coupling, the cycloaddition of the alkyne-terminated polymers with N_3-HEC was proven unambiguously by ^{15}N solid state NMR spectroscopy. This indicates the utility of ^{15}N labelling for probing the coupling efficiency of CuAAC reactions when employed in 'grafting to' processes with cellulosic substrates.

Introduction

With an annual production of ~$10^{11\text{-}12}$ tonnes[1-3], cellulose is the most abundant bio-polymer on Earth and the major compound in the cell wall of plants. Cellulose possesses a supra-molecular structure which results from the presence of two H-bonding networks. The intramolecular H-bonding network occurs between hydroxyl groups within the same chain whereas the intermolecular H-bonding results from the interaction of the hydroxyl groups between chains. These networks are responsible for the main drawback of cellulose, which is its poor solubility in both organic and aqueous solvents[3-6]. This results in difficulties in processing cellulose, limiting its industrial applications. Chemical modification of cellulose is possible due to the presence of three hydroxyl groups at C2, C3 and C6 positions, resulting in derivatives such as hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC) and carboxymethyl cellulose (CMC) where the degree of substitution (DS) ranges from 0 to 3[7, 8]. HEC (Figure 1) is obtained by etherification with ethylene oxide and is defined by both the DS and the molecular substitution (MS), which represents the length of the ethyleneoxide side chain. This addition to the cellulose backbone disrupts the H-bonding networks resulting in solubilisation in polar solvents such as dimethylsulfoxide (DMSO) and water. HEC has been used extensively as an emulsifier, a stabiliser, thickeners and cosmetic film-formers in the formulation of hair and skin products[9-11].

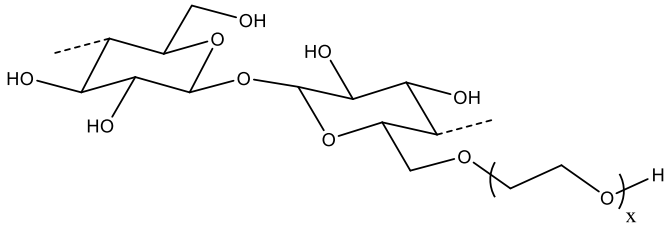


Fig. 1: Chemical structure of HEC

A promising method for extending the range of industrial uses of cellulose is the preparation of graft copolymers. This allows the creation of new materials with the properties of synthetic polymers but which are obtained (at least in part) from a renewable feedstock. Three grafting approaches[8, 12, 13] can be employed: "grafting to", "grafting from" and "grafting through". The latter consists of polymerisation of monomeric

species onto macro-monomers, and has been sparsely investigated in respect of cellulose derivatives. "Grafting from" is commonly used, where synthetic polymer chains are grown from sites on the cellulose backbone. The cellulose backbone can be activated via a range of processes including single electron transfer[14], hydrogen abstraction[15, 16], irradiation[17, 18] or other methods[8, 12, 19]. From the resulting macro-initiator sites, polymer chains can be grown producing the graft copolymer. The "grafting to" approach involves the chemical conjugation of a pre-prepared polymer to the cellulose backbone. The conjugation is possible through the presence on each polymer of complementary functional groups which undergo efficient coupling reactions such as thiol-ene[20], hetero-Diels-Alder[21, 22] or copper catalysed azide-alkyne cycloaddition (CuAAC)[23-25].

Graft copolymerisation of cellulosic derivatives has mainly been conducted by the "grafting from" method using a cellulosic macro-initiator[26]. Hansson et al.[27] used ATRP to prepare cellulose-g-PMMA by grafting from a macroinitiator immobilised on a solid cellulose substrate. Semsarilar et al.[28] were the first to report the use of RAFT in combination with a cellulosic material; a hydroxypropyl cellulose (HPC) macro-CTA was used in the RAFT polymerisation of N-isopropylacrylamide (NIPAAM) and ethyl acrylate. Compared to the "grafting from" approach, however, "grafting to" has the significant advantage that it allows the full characterisation of both the grafts and the cellulose backbone prior to the coupling reaction. This method has been used to prepare a range of cellulosic graft copolymers. Hansson et al.[27] coupled alkyne-terminated PMMA to a solid cellulose substrate, prior-functionalized with azide groups, using CuAAC 'click' chemistry. Goldman et al.[21] grafted RAFT-polymerised poly(isobornyl acrylate) to a cellulose surface using a hetero-Diels-Alder cycloaddition and recently, Xiao et al.[29] used RAFT polymerisation in combination with a thiol-ene 'click' reaction to prepare methyl cellulose-g-poly(vinyl acetate).

In our work we sought to prepare well-defined graft copolymers of HEC using a homogeneous (solution) coupling approach. We chose RAFT polymerisation to prepare synthetic polymers because of its versatility in the range of monomers that are able to be polymerised in a controlled manner. Alkyne-containing chain transfer agents[30] suitable for preparing 'clickable' polymers from a range of monomers including N-vinyl-pyrrolidone, vinyl acetate, styrene and n-butyl acrylate[31-35], were employed. N-vinylpyrrolidone (NVP) was chosen to exemplify our approach because it is a challenge to polymerise by other CRP methods, such as ATRP. In order to demonstrate the versatility of our method, alkyne-terminated PNIPAAM was also prepared by RAFT polymerisation. Copper catalysed azide-alkyne cycloaddition (CuAAC) was chosen to 'click' alkyne-terminated polymers to azido-HEC (N_3-HEC). The HEC derivatives had limited solubility in common solvents, consequently characterisation was performed mainly using solid state methods. Indirect or weak confirmation of the CuAAC reaction was provided by solid state ^{13}C NMR and FTIR spectroscopic investigations of the poorly soluble graft copolymer products. Partial ^{15}N-labelling of N_3-HEC, however, allowed unambiguous verification of the coupling reaction using solid state ^{15}N NMR spectroscopy and is therefore a powerful tool to confirm the success of 'grafting to' processes involving CuAAC reactions with cellulosic substrates.

Experimental

Materials and methods

All chemicals were purchased from Sigma Aldrich and were used as received. Solution state NMR spectra were recorded using a Bruker Advance 400 spectrometer at 400.13 MHz (^1H) and 100.60 MHz (^{13}C). For solid state NMR spectroscopy, a Varian VNMRS spectrometer with a 9.4 T magnet was used and the ^{13}C (100.562 MHz) and ^{15}N (40.527 MHz) experiments were run using the cross polarisation method. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer. Molecular weight data were obtained using triple detection size exclusion chromatography (SEC) on a Viscotek TDA 302 with refractive index, viscosity and light scattering detectors and 2 × 300mL PLgel 5 μm mixed C columns. Dimethylformamide (DMF) was used as the eluent at a flow rate of 1.0 mL/min and at a constant temperature of 70 ºC.

Synthesis of azido-hydroxyethyl cellulose (N_3-HEC)

The procedure followed that reported in the literature[36]. In a round-bottomed flask fitted with a condenser, 2-hydroxyethyl cellulose 1 (M_w =90 000 g/mol, MS =2.5, 2.5 g, 9.19×10^{-3} mol, 1 eq), sodium azide (3.8 g, 5.85×10^{-2} mol, 6 eq.) and ^{15}N-labelled sodium azide $Na^{15}N_3$ (0.1 g, 1.54×10^{-3} mol, 0.1 eq) were dissolved in DMF (100 mL). The mixture was heated to 80 °C for 1 h in order to dissolve HEC. The mixture was cooled to room temperature and carbon tetrabromide (14.3 g, 4.31×10^{-2} mol, 5 eq) was added. Triphenylphosphine (11.8 g, 4.50×10^{-2} mol, 5 eq) dissolved in DMF (12.5 mL) was added carefully to the HEC mixture. The reaction was then left for 24 h at room temperature under magnetic stirring. The product was precipitated by addition of toluene (500 mL) and collected by filtration. The solid was dissolved in DMF (50 mL) and re-precipitated in diethyl ether (500 mL). After being filtered, the solid was washed with acetone (100 mL) and dried under vacuum at 50 °C overnight. The product **2** was obtained as a light yellow solid in quantitative yield (3.1 g). The product was characterised using solid state ^{13}C, ^{15}N CP-MAS NMR and FT-IR spectroscopies.

Synthesis of O-ethyl S-prop-2-ynyl carbonodithiolate (5)

The procedure follows that reported in the literature[31]. In a one-neck round-bottomed flask, propargyl bromide solution **3** (80% wt. in toluene, 1.02 g, 8.57×10^{-3} mol) and potassium ethyl xanthogenate **4** (1 g, 6.24×10^{-3} mol) were dissolved in THF (10 mL). The flask was covered with aluminium foil and the reaction was run overnight at room temperature. THF (100 mL) was added and the mixture was filtered to remove KOH. The

excess solvent was evaporated and distilled water (10 mL) was added to the residues. The product was extracted with diethyl ether (3×30 mL). The diethyl ether was removed and the final product **5** was purified via column chromatography using pentane as eluent and dried under vacuum overnight. The product was obtained as a pale yellow oil in a yield of 48% (0.48 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H (ppm) 1.4 (t, J 7.0 Hz, 3H, C**H$_3$**-CH$_2$-O), 3.2 (t, J =2.6 Hz, 1H, C**H**≡C-), 4.0 (d, J =2.7 Hz, 2H, CH≡C-C**H$_2$**-), 4.6 (q, J =7.0 Hz, 2H, -C**H$_2$**-O); ^{13}C NMR (400 MHz, DMSO-d_6): δ_C (ppm) 13.5 (**C**H$_3$-CH$_2$-O-), 23.5 (-**C**H$_2$-S-), 70.6 (-**C**H$_2$-O-), 74.1 (**C**H≡C-), 78.6 (CH≡**C**-), 211.8 (-**C**=S).

Synthesis of Alkyne-terminated trithiocarbonate (8)

The procedure follows that developed by Ranjan and co-worker[33]. In a one-neck round-bottomed flask, trithiocarbonate CTA **6** (1 g, 2.74×10^{-3} mol, 1 eq.), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) (0.783g, 5.04×10^{-3} mol, 2eq.) and 4-(dimethylamino)pyridine (DMAP) (0.5 g, 4.09×10^{-3} mol, 2 eq.) were dissolved in dichloromethane (10 mL) and the mixture was purged with nitrogen for 10 min. Propargyl alcohol **7** (0.5 mL, 8.59×10^{-3} mol, 3 eq.) was added and the mixture was stirred overnight at room temperature under positive N_2 pressure. The flask was opened to air and the product **8** was extracted by washing with each of the following solvents (3×30 mL); dilute aqueous HCl, distilled water and brine solution (3.5% w/w NaCl). After concentration under vacuum, the product **8** was obtained as a viscous yellow liquid in a yield of 87% (0.95 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 0.8 (t, 3H, J =7.0 Hz, C**H$_3$**-CH$_2$-), 1.19-1.66 (m, 20H, CH$_3$-(C**H$_2$**)$_{10}$-), 1.6 (s, 6H, -S-C(C**H$_3$**)$_2$-), 2.4 (t, 1H, J =2.4 Hz, -C≡C**H**), 3.2 (t, 2H, J =7.5, -C**H$_2$**-S-), 4.6 (d, 2H, J =2.5 Hz, -O-C**H$_2$**-); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 14.3 (**C**H$_3$-), 22.8 (CH$_3$-**C**H$_2$-), 25.4 (C-(**C**H$_3$)$_2$-) , 28-32 (9C, CH$_3$-CH$_2$-(**C**H$_2$)$_9$-) , 37.1 (-**C**H$_2$-S-), 53.5 (-O-**C**H$_2$-), 55.7 (-**C**(CH$_3$)$_2$-), 75.2 (-C≡**C**H), 77.4 (-**C**≡CH), 172.5 (-**C**=O).

RAFT polymerisation of N-vinylpyrrolidone

In a one-neck round-bottomed flask, O-ethyl S-prop-2-ynyl carbonodithiolate **5** (0.50 g, 3.05×10^{-3} mol, 1 eq.), AIBN (0.35 g, 2.13×10^{-3} mol, 0.7 eq.) and N-vinyl-pyrrolidone **9** (3.44 g, 3.09×10^{-2} mol, 10 eq.) were dissolved in toluene (20 mL). The mixture was purged under N_2 for 10 min. The flask was sealed and the reaction was run overnight at 70 °C. The mixture was then cooled to room temperature and the product was precipitated in diethyl ether. The product was collected by filtration and dried overnight under vacuum at 40 °C. The product 10 was obtained as a white powder in quantitative yield (3.30 g). The polymer was characterized using solution state ^{1}H NMR spectroscopy and SEC. NMR spectra were recorded in $CDCl_3$ and the number average molecular weight (M_n) and the dispersity ($Đ_M$) were determined using SEC with conventional calibration (PMMA standards). Solid state ^{15}N CP-MAS NMR (40.52 MHz): δN (ppm) -253.8.

RAFT polymerisation of N-isopropyl acrylamide

In a one-neck round-bottomed flask, alkyne-terminated CTA **8** (0.4 g, 9.93×10^{-4} mol, 1 eq.), N-isopropyl acrylamide 11 (1.12g, 9.90×10^{-3} mol, 10 eq.), and AIBN (0.02 g, 1.21×10^{-4} mol, 0.12 eq.) were dissolved in 1,4-dioxane (10 mL). The flask was purged under N_2 for 15 min and, once sealed, the polymerisation was run overnight at 60 °C. The polymer was precipitated in hexane (100 mL) and recovered by filtration. The product **12** was dried overnight under vacuum at 40 °C and was obtained as a white powder in a yield of 87% (1.12 g). The polymer was characterised using solution state ^{1}H NMR spectroscopy and SEC. NMR spectra were recorded in $CDCl_3$ and the M_n and $Đ_M$ were determined using SEC with conventional calibration (PMMA standards).

Copper-catalysed azide-alkyne cycloaddition (CuAAC) between N_3-HEC and 5

In a one-neck round-bottomed flask, N_3-HEC **2** (0.5 g, 1.7×10^{-3} mol, 1 eq.), O-ethyl S-prop-2-ynyl carbonodithiolate (0.7 g, 4.4×10^{-3} mol, 3 eq.) **5**, sodium L-ascorbate (0.24 g, 3.3×10^{-4} mol, 0.2 eq.), copper (II) sulfate pentahydrate (0.15 g, 1.7×10^{-4} mol, 0.1 eq.) and N,N,N′,N′-tetramethylethylenediamine (0.07 g, 1.7×10^{-4} mol, 0.1 eq) were dissolved in DMF (10 mL). The flask was heated at 30 °C for 24 h, the mixture was cooled to room temperature and the products were precipitated with chloroform (100 mL). The solid was then filtered and was washed in acetone (~20 mL) under stirring in order to remove the unreacted RAFT agent. The product **13** was then filtered and dried overnight under vacuum at 50 °C. The product was obtained in near quantitative yield (0.7 g). The macro-CTA was characterised using solid state (^{13}C and ^{15}N) CP-MAS NMR spectroscopies and FTIR spectroscopy.

The same procedure has been repeated without the use of copper sulfate pentahydrate in order to investigate the regioselectivity of the reaction. The resulting product obtained in a yield of 66% (0.33 g) was characterised using (^{13}C and ^{15}N) CP-MAS NMR and FTIR spectroscopies.

Copper-catalysed azide-alkyne cycloaddition (CuAAC) between N_3-HEC and alkyne-terminated polymers

In a round-bottomed flask fitted with a drying tube, N_3-g-HEC 2 (0.5 g, 1.7×10^{-3} mol, 1 eq.), alkyne-terminated poly(N-vinylpyrrolidone) **10** (2 eq, 0.3 eq and 0.5 eq), sodium L-ascorbate (0.57 g, 2.88×10^{-3} mol, 2 eq.), copper (II) sulfate pentahydrate (0.36 g, 1.44×10^{-3} mol, 1 eq.) and N,N,N′,N′-tetramethylethylenediamine (0.17 g, 1.46×10^{-3} mol, 1 eq) were dissolved in DMF (20 mL). The flask was heated at 30 °C for 24 h. The mixture was cooled to room temperature and polymeric materials were precipitated with diethyl ether (200 mL). The solid was then collected by filtration and was washed for 24 h in water (~20 mL) under stirring in order to remove the unreacted PVP chains. The product **14** was then filtered and dried overnight under vacuum at 50 °C. The product was obtained in a yield of 40% mass. The graft-copolymers were

characterised using solid state ^{13}C and ^{15}N CP-MAS NMR and FTIR spectroscopies.

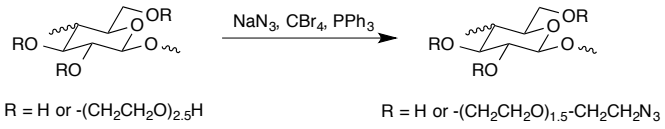
The same procedure has been followed for coupling PNIPAAM **12** (1.06 g, 6.91×10^{-4} mol, 3 eq.) to N_3-HEC **2** (0.06 g, 2.02×10^{-4} mol, 1 eq.) and the resulting product 15 was obtained in quantitative yield (0.47 g). The polymer was characterised using solid state ^{13}C, ^{15}N CP-MAS NMR and FTIR spectroscopies.

Results and discussion

To prepare HEC graft copolymers by a 'grafting to' strategy using CuAAC coupling, we require HEC with one coupling partner in the side-chain and polymers with the other coupling partner as an end group. We decided to install the alkyne at the polymer chain end using alkyne-functionalised chain transfer agents. These would be coupled to azide-substituted HEC (N_3-HEC).

Synthesis of azido-hydroxyethyl cellulose (N_3-HEC)

N_3-HEC was prepared from HEC in a one-step procedure in the presence of carbon tetrabromide and triphenyl phosphine (Scheme 1)[36]. In order to aid the detection of nitrogen by ^{15}N NMR spectroscopy, sodium azide was doped with $Na^{15}N_3$.



Scheme 1. Synthesis of partially ^{15}N-labelled N_3-HEC

The resulting azido-HEC product was found to have poor solubility in common solvents, therefore characterisation was performed in the solid state. The solid state ^{13}C NMR spectrum (Figure 2b) shows signals at ~103 ppm, 83 ppm, 74ppm, 71 ppm and 52 ppm. The signals at ~103 ppm and 81 ppm are typical of HEC and are assigned respectively to the anomeric carbon (C1) and the carbon at the C4 position. The signals δ_C ~74 ppm are assigned to the set of carbons at C2, C3 and C5 positions and the peak δ_C ~71 ppm is assigned to the carbons of the ethylene oxide side chain. The signal at ~52 ppm is assigned to the $-CH_2-N_3$ and the loss of the signal at ~62 ppm in the HEC spectrum commonly assigned to the C6 (R=H) and the final carbon of the ethylene oxide side chain indicates complete functionalization of the primary alcohol of HEC with NaN_3.

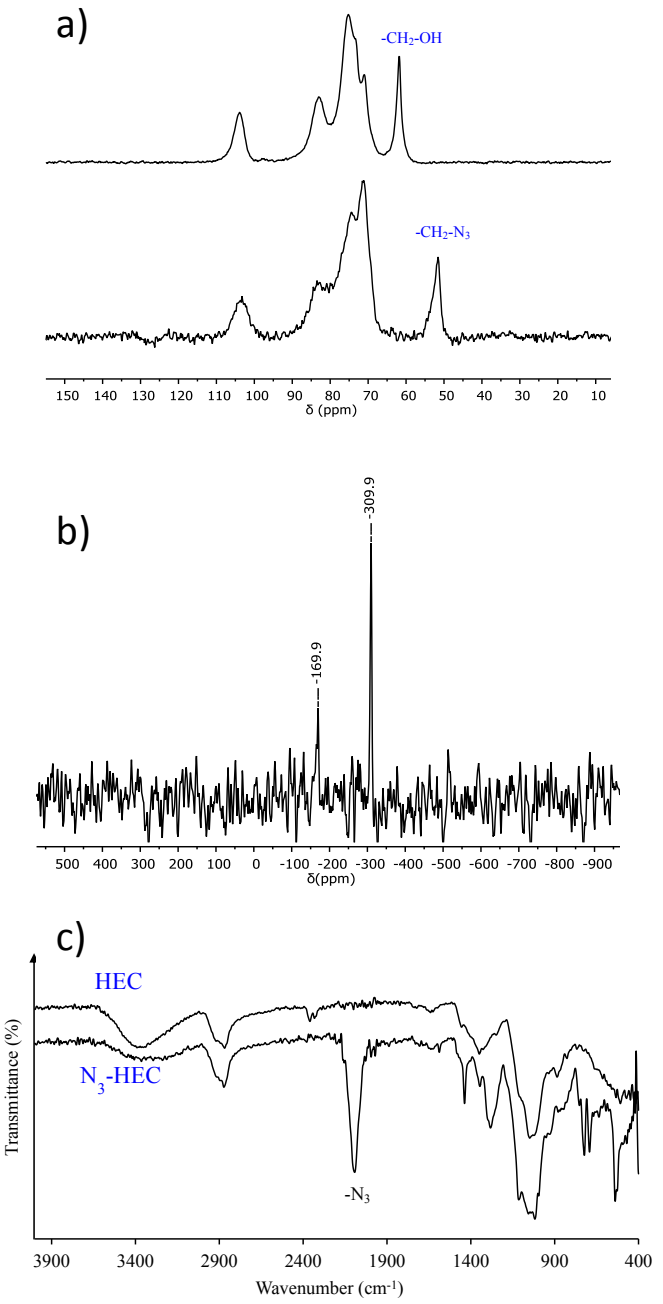


Fig. 2. Characterisation of N_3-HEC: a) solid state ^{13}C CP-MAS NMR spectra of HEC **(top)** and N_3-HEC**; b) s**olid state ^{15}N CP-MAS NMR spectrum of N_3-HEC**; c)** FTIR spectra of HEC **(top)** and N_3-HEC**.**

The solid state ^{15}N NMR spectrum (Figure 2b) shows two signals at ~-310 and -170 ppm which are characteristic respectively of $-CH_2-N_\alpha=N_\beta=N_\gamma$ and $-CH_2-N_\alpha=N_\beta=N_\gamma$[37]. The labelled NaN_3 is labelled at N_α and N_γ only, and this explains why N_β was not detected. In the cross polarisation method, the detection of nitrogen is obtained from the transfer of energy from protons to the nitrogen. Thus, the more protons environments, there are near to the nitrogen and the closer these proton are to the nitrogen, the easier will be their detection. In N_3-HEC, N_α is directly attached to a methylene group which

represents also the closest source of protons for N_γ. The excitation energy received by N_α is greater than that received by N_γ, resulting in an easier detection of N_α which is demonstrated by a higher intensity of the signal δ_N ~-310 ppm assigned to N_α. The FTIR spectrum of N_3-HEC (Figure 2c) shows a strong absorbance band at 2100 cm^{-1}, characteristic of the presence of an azide group and a decrease of the absorbance band at 3400 cm^{-1} indicating the loss of some hydroxyl groups, reinforcing the evidence of the functionalization of the primary alcohol with sodium azide.

Preparation of alkyne-terminated polymers

The chain transfer agent (CTA) O-ethyl-S-prop-2-ynyl carbonodithiolate (**5**) was prepared following a reported procedure[31]. Propargyl bromide was reacted with potassium ethyl xanthogenate overnight at room temperature to obtain **5** in a yield of 50% (Supplementary Information, Scheme S1). The CTA **5** was then used to polymerise N-vinylpyrrolidone (NVP) in a controlled manner to give PVP chains with alkyne end groups (Supplementary Information, Scheme S3). In the literature[38-42], both xanthate and dithiocarbamate chain transfer agents were shown to be good candidates for polymerising NVP, a monomer that is a member of the "less activated monomers" (LAM) class described by Keddie et al.[43] Furthermore, Patel et al.[44] and Akeroyd et al.[31] reported the preparation of alkyne-terminated RAFT agents which were used for preparing "macro-RAFT agents" or clickable polymers respectively.

The ratio RAFT agent : initiator (AIBN) was optimised to obtain relatively high conversion and good control of the polymerisation, and we found that 1:0.7 was a good compromise, giving a monomer conversion and a $Đ_M$ of 80% and 1.4 respectively. The degree of polymerisation ($DP_{targeted}$ =10) was kept low in order to facilitate the spectroscopic detection of the HEC backbone and PVP in the final graft-copolymer HEC-g-PVP. At the end of the reaction, 80% monomer conversion was determined and PVP was isolated by precipitation with diethyl ether in near-quantitative yield. The polymer was then characterised using NMR spectroscopy and SEC. The ^{1}H NMR spectrum of PVP (Supplementary Information, Figure S1) shows signals characteristic of the protons of the pyrrolidone ring as well as peaks that can be assigned to the chain end -CH_3 and the methylene adjacent to the alkyne. Furthermore, the signal at δ_H ~4.6 ppm is assigned to the non-alkyne end group CH_2-O. The DP was estimated using the integral of the signal at δ_H ~4.6 ppm, which gave an $M_{n\ NMR}$ value of 1,500 g/mol. This is in good agreement with the targeted DP of 10.

The SEC results (Supplementary Information, Figure S2) indicate a number average molecular weight ($M_{n\ SEC}$) of 1 180 g/mol and a dispersity ($Đ_M$) of 1.4, calculated with conventional calibration using PMMA standards. The $M_{n\ SEC}$ value is not representative of the true value because of a non-negligible difference of the hydrodynamic volume of PMMA standards and PVP chains. However, the $Đ_M$ value is characteristic of the true molecular weight distribution. The $Đ_M$ value of 1.4 indicates a relatively good control of the polymerisation, given the low DP.

An alkyne-terminated trithiocarbonate **8** was synthesised from CTA **6** and propargyl alcohol in a yield of 87% (Supplementary Information, Scheme S2). NIPAAM was successfully polymerised using **8** at a 1:0.12 ratio of initiator to chain transfer agent (Supplementary Information, Scheme S4). The alkyne-ended pNIPAAM was obtained with a monomer conversion of 90% determined by ^{1}H NMR spectroscopy. In the ^{1}H NMR spectrum (Supplementary Information, Figure S3), some of the transfer chain signals are detected with the peak at δ_H ~0.8 ppm assigned to the methyl group of the chain end, δ_H ~1.2 ppm to the gem-dimethyl groups, δ_H ~1.8 ppm to the -C≡CH, δ_H ~3.2 ppm to the -CH_2-S-, δ_H ~4.6 ppm to the -O-CH_2- and δ_H ~6 ppm to the -NH group. From the integration of the signal of the end group at δ_H ~4.6 ppm compared to the methine group at δ_H ~3.9 ppm characteristic of PNIPAAM, a degree of polymerisation (DP_{NMR}) of ~10 was estimated and this corroborated with the calculated monomer conversion. The $Đ_M$ measured by SEC was found to be 1.2, confirming the control of the polymerisation. Because of a very low targeted DP, the calculation of the molecular weight using SEC was not reliable regardless of the detector used. However, the confirmation of a narrow molecular weight distribution permitted the use of the calculated DP_{NMR} to give an approximation of the molecular weight as ~1,400 g/mol. Xanthate CTA **5** did not give good control of NIPAAM RAFT polymerisation (($Đ_M$ =1.5; Supplementary Information, Figure S4)

Copper Catalysed Azide-alkyne Cycloaddition (CuAAC)

In order to test the efficiency of the CuAAC reaction, coupling was attempted initially with alkyne-CTA **5** (Supplementary Information, Scheme S5). It was expected that the low molar mass of **5** would aid characterisation of the click product. CTA-functionalized HEC was prepared via CuAAC in 100% yield assuming complete coupling. Again due to poor solubility of the coupling product in deuterated NMR solvents, it was characterised in the solid state using FTIR and solid state NMR spectroscopies.

The solid state ^{13}C NMR spectrum (Figure 3a) shows a large band of peaks between 71 and 81 ppm which are assigned to the carbons of the HEC backbone. The peak at 103.2 ppm is assigned to the carbon at the C1 position, and the peak at 51.9 ppm is assigned to the carbon directly attached to the nitrogen of the triazole ring. Comparing the product to the N_3-HEC spectrum (Figure 2), the chemical shift of this carbon is the same as that of $\underline{CH_2}$-N_3. However, the click reaction is demonstrated by the presence of peaks at 124.0 and 123.2 ppm which are assigned to the carbons in the triazole ring. Furthermore, the peak at 15.0 ppm and the broad peak at 32.4 ppm are assigned respectively to the methyl and methylene of the RAFT agent. The FTIR spectrum (Figure 3b) showed a strong decrease of the absorption band at 2100 cm^{-1} assigned to the azide groups, which provides indirect evidence of the functionalization reaction (disappearance of azide groups). The

coupling between the azide and the transfer agent however appears not to be complete.

To provide further evidence of the success of the 'click' reaction, solid state ^{15}N NMR spectroscopy was employed. In the ^{15}N NMR spectrum (Figure 3c), only one peak at δ_N ~-134 ppm is present and is assigned to the N_α of the triazole cycloaddition product.

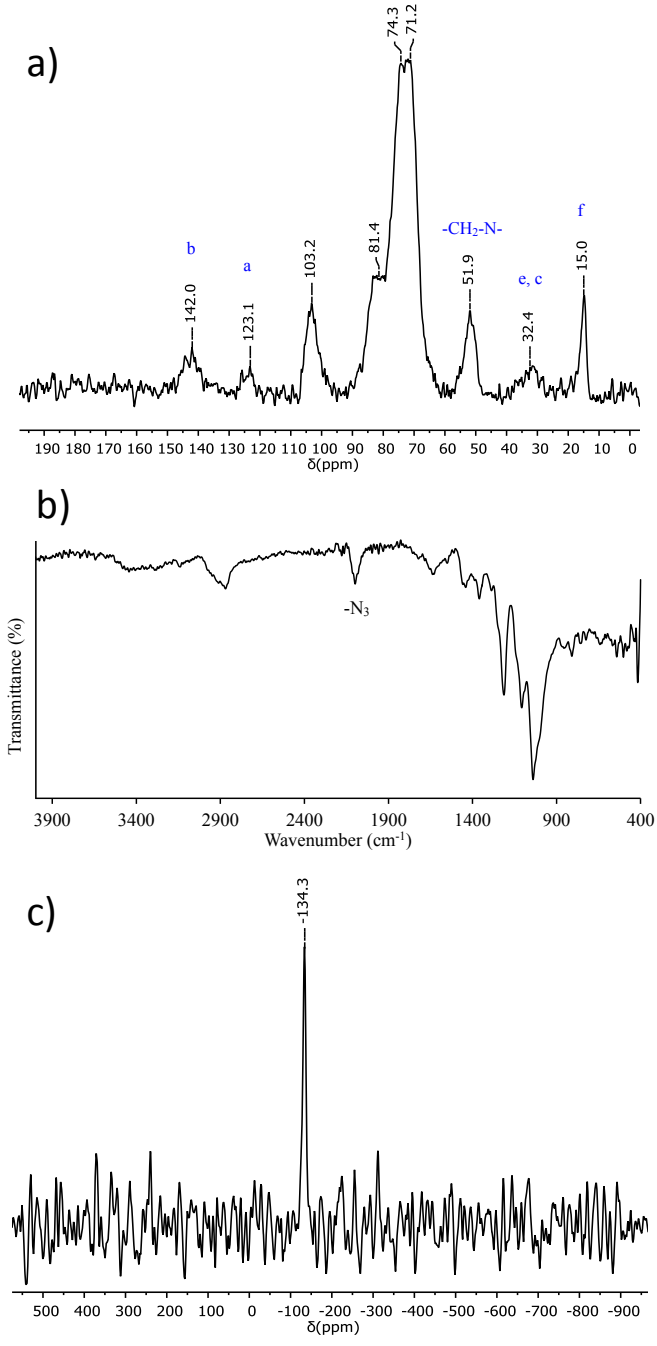


Fig. 3. Characterisation of CTA-functionalised HEC: a) solid state ^{13}C CP-MAS NMR spectrum; b) FTIR spectrum; c) solid state ^{15}N CP-MAS NMR spectrum. Peak labels in a) are: a,b – triazole ring; c, e and f – xanthate CTA (see Supplementary Information for full assignment).

The large change in the chemical shift of N_α from -310 ppm to -134 ppm suggests complete reaction of N_3 with the alkyne group of the transfer agent, however, because the FTIR spectrum showed the presence of unreacted N_3, we suppose that the peak at -310 ppm corresponding to unreacted N_α was too weak and thus was undetectable by NMR. Compared to the spectrum of N_3-HEC (Figure 2), the ratio of the signal of N_α to noise is lower due to the change of the environment in terms of protons and the change in the symmetry of the environment. The peak present at -170 ppm in the ^{15}N spectrum of N_3-HEC (Figure 2) and assigned to N_γ is absent from the ^{15}N spectrum of the macro-CTA because the ratio of signal N_γ to noise has decreased, resulting in an inability to detect it. This peak was expected to shift to approximately -20 ppm according to studies carried by Corredor et al.[45] N_3-HEC was therefore quasi-completely functionalised with the RAFT agent using a CuAAC reaction resulting in the formation of a CTA-functionalised HEC backbone. This allows us to identify the correct conditions for the click reaction and also to determine the ^{15}N NMR chemical shifts of the triazole product, which will be of great value in confirming the grafting of alkyne-ended polymers to N_3-HEC.

The CuAAC reaction was next used to couple PVP_{10} to N_3-HEC, forming the graft-copolymer HEC-g-PVP_{10} (Supplementary Information, Scheme S5). A chain length of 10 repeat units was chosen because this aided the characterisation of the graft copolymer and the properties of HEC should be retained. To examine the scope of the grafting process, ratios of PVP chain to azide groups equal to 1:5, 1:3 and 1:1 were chosen. The graft-copolymer and possibly some unreacted PVP_{10} chains were precipitated with diethyl ether. Ungrafted PVP_{10} chains were then separated from HEC-g-PVP_{10} by extensive washing in water (PVP_{10} chains are water soluble whereas HEC-g-PVP_{10} is not). Based on the theoretical ratio of PVP chains to azide groups, HEC-g-PVP_{10} was obtained in a yield of ~40%.

In the ^{13}C NMR spectrum of HEC-g-PVP_{10} with a ratio of PVP to N_3 equal to 1:5 (Figure 4b), peaks characteristic of both HEC and PVP were detected. The peak at 51 ppm is assigned to the carbon at the C10 position, the large band between 60 ppm to 90 ppm is assigned to the carbons of the HEC ring and the ethyleneoxide side chain and the peak at 104 ppm is assigned to the anomeric centre (C1) (structure numbering given in Supplementary Information, Figure S5). The presence of PVP is demonstrated by the peaks at 19.3 ppm, 32.6 ppm, 43.8 ppm and 176.7 ppm which are assigned respectively to the pyrrolidone ring and the carbonyl group. When the ratio of PVP to -N_3 is increased to 1:3 (Figure 4c), the intensity of the peaks assigned to PVP increases whereas the intensity of the peaks characteristic of HEC decreases, due to the increase of the density of grafting of HEC with PVP_{10}. Increasing the graft-density to attempt to achieve complete functionalization of N_3-HEC with PVP resulted in an almost complete disappearance of HEC signals (Figure 4d). The triazole carbons resulting from the cycloaddition between PVP_{10} and N_3-HEC were not

detected because of their low concentration within the structure. The NMR experiments indicate the presence of PVP and HEC in the product, and the signals of the HEC backbone decreased with increasing grafting density. However, definitive evidence of the coupling is not demonstrated using these NMR experiments.

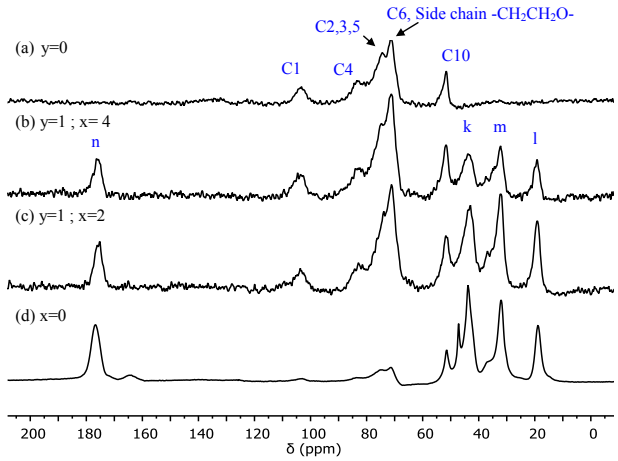


Fig. 4. Solid state ^{13}C CP-MAS NMR spectra of (a) N_3-HEC, (b) HEC-g-PVP_{10} (1:5 PVP:–N_3), (c) HEC-g-PVP_{10} (1:3 PVP:–N_3), (d) HEC-g-PVP_{10} (1:1 PVP:–N_3). Theoretical density of grafting denoted by x and y where x = azido-HEC units and y = PVP grafted units. Protons of the pyrrolidone ring of PVP are labelled k, l, m and n (see Supplementary Information for assignment).

In the FTIR spectrum of N_3-HEC (Figure 5a), an absorption band at ~2100 cm^{-1} typical of azide groups is present. Coupling PVP_{10} with N_3-HEC at a ratio PVP:N_3 of 1:5 (Figure 5b) caused the intensity of the band at 2100 cm^{-1} to decrease and a band at ~1700 cm^{-1} appears which is assigned to the carbonyl group in PVP_{10}. Increasing the graft-density of PVP to N_3-HEC (Figure 5c), the band at 2100 cm^{-1} decreases whereas the band at 1700 cm^{-1} increases. The complete disappearance of the band at 2100 cm^{-1} when the ratio PVP:N_3 was 2:1 (Figure 5d) indicates complete functionalization of N_3-HEC with PVP_{10}. These spectra indicate disappearance of the azide band which implies successful CuAAC reaction but no direct evidence of the coupling is presented.

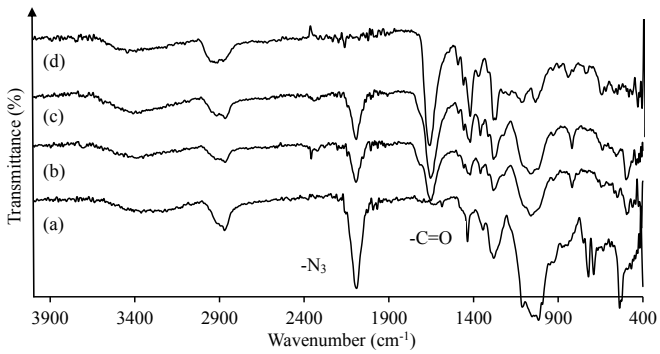


Fig. 5. FT-IR spectra of (a) N_3-HEC, (b) HEC-g-PVP_{10} (1:5 PVP:–N_3), (c) HEC-g-PVP_{10} (1:3 PVP:–N_3), (d) HEC-g-PVP_{10} (2:1 PVP:–N_3).

To try to demonstrate more clearly the CuAAC coupling between PVP_{10} and N_3-HEC, partially ^{15}N-labelled N_3-HEC was prepared. In the ^{15}N NMR spectrum of N_3-HEC (Figure 6a), the two peaks at ~-310 and -170 ppm are assigned to N_α and N_γ. When PVP_{10} was coupled to N_3-HEC at a ratio PVP:N_3 equal to 1:5 (Figure 6b), a peak at -256.3 ppm appeared which is assigned to the unlabelled nitrogen in the pyrrolidone ring (the ^{15}N signal for PVP_{10} occurs at -253.8 ppm). Increasing the PVP:N_3 ratio to 1:3 (Figure 6c) resulted in the detection of an additional signal at ~-140 ppm which is assigned to N_α after its cyclisation with PVP_{10}. The peaks of unfunctionalized N_α and N_γ are still present in the spectrum but their intensities are decreased compared to the signal of nitrogen in PVP, indicating an increase of the functionalization of N_3-HEC with PVP_{10}.

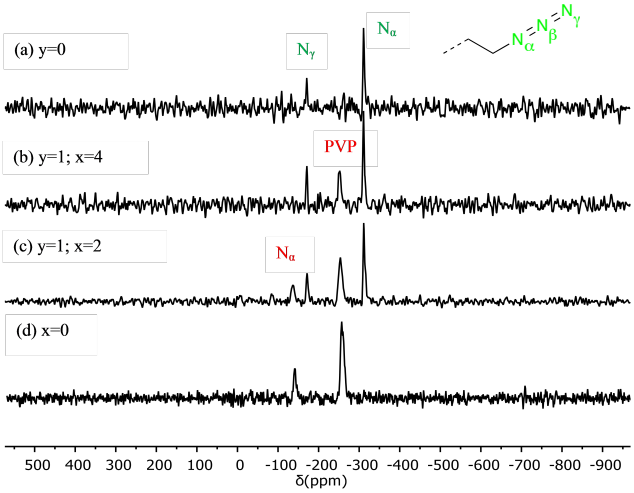


Fig. 6. Solid state ^{15}N CP-MAS NMR spectra (a) N_3-HEC, (b) HEC-g-PVP_{10} (1:5 PVP:–N_3), (c) HEC-g-PVP_{10} (1:3 PVP:–N_3), (d) HEC-g-PVP_{10} (1:1 PVP:–N_3). Theoretical density of grafting denoted by x and y where x = azido-HEC units and y = PVP grafted units. Inset above a) shows labelling of azide nitrogens.

For the spectrum of HEC-g-PVP_{10} prepared from a ratio PVP:N_3 equal to 2:1 (Figure 6d), two signals at -256.3 ppm and -140.4 ppm are detected and are assigned respectively to the nitrogen in the pyrrolidone ring and the triazole N_α after the cycloaddition process. The intensity of the signal of N_γ in N_3-HEC decreased when the graft-density increased, however, no N_γ signal was detected after cyclisation. The complete loss of both signals at -310 ppm and -170 ppm, assigned respectively to N_α and N_γ in N_3-HEC, demonstrates complete coupling between N_3-HEC and PVP_{10} and this corroborates with the discussion of the ^{13}C NMR and FTIR spectra of HEC-g-PVP_{10}.

In order to demonstrate the versatility of our grafting method, $PNIPAAM_{10}$ synthesised with an alkyne-terminated trithiocarbonate CTA was coupled to N_3-HEC using identical conditions to those described in the previous section (Supplementary Information, Scheme S6). PNIPAAM peaks were detected in the solid state ^{13}C NMR spectrum of the graft copolymer (Figure 7a). Additional weak peaks (Figure 7a inset) are observed when increasing the intensity of signals and these are assigned either to the cellulose backbone or the chain

transfer agent. The peaks at 14.7 ppm and 242.4 ppm are assigned to the methyl and thiocarbonate group of the RAFT agent. Furthermore, bands at ~71 and ~107 ppm are characteristic of the NMR signals commonly observed for cellulosic materials (Figure 7b), and were assigned respectively to the set of carbons at C2 to C6 positions and to the anomeric centre (C1). The cycloaddition between PNIPAAM and N_3-HEC was suggested by the presence of weak signals at ~125 and 143 ppm which are assigned to triazole ring carbons. The FTIR spectrum (Supplementary Information, Figure S6) showed a complete disappearance of the azide band suggesting successful reaction with the alkyne group at the chain end of $PNIPAAM_{10}$.

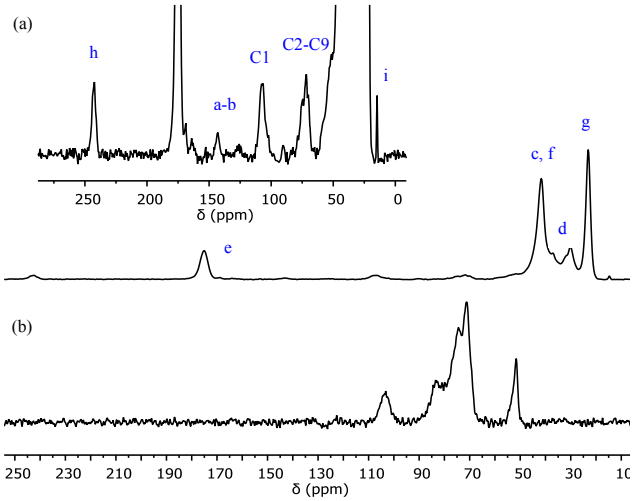


Fig. 7. Solid state ^{13}C NMR spectrum of (a) HEC-g-$PNIPAAM_{10}$ and (b) N_3-HEC.

The solid state ^{15}N NMR spectrum (Figure 8a) of the graft-copolymers shows two signals -134.8 ppm and -244 ppm which are respectively assigned to the labelled nitrogen $^{15}N_\alpha$ of N_3-HEC after the cycloaddition and the unlabelled nitrogen of PNIPAAM. The difference in intensity between the two peaks is explained by the concentration of each within the structure; there is only one partially labelled N_α per 10 nitrogens of PNIPAAM in the structure, resulting in a low intensity of the signal of N_α. Furthermore, the signals of N_α and N_γ of N_3-HEC (Figure 8b) disappeared after the cycloaddition, indicating complete functionalization.

Conclusions

Graft copolymers of hydroxyethyl cellulose (HEC) and either poly(N-vinylpyrrolidone) (PVP) or poly(N-isopropylacrylamide) (PNIPAAM) have been prepared by a 'grafting to' approach using CuAAC coupling. To achieve this, azido-substituted HEC and alkyne-terminated RAFT polymers were prepared. A degree of polymerisation of 10 was targeted for each polymer so that the final graft copolymer would retain a significant mass fraction of HEC after coupling. Both RAFT polymers were synthesised successfully with monomer conversions of 80-90% and $Đ_M$ values ranging from 1.2 (PNIPAAM) to 1.4 (PVP). HEC-g-PVP_{10} and HEC-g-$PNIPAAM_{10}$ hybrid materials with different graft densities were prepared by CuAAC coupling using different ratios of synthetic polymer to azide groups. Solid state ^{13}C NMR and FTIR spectroscopies provided weak or indirect evidence of coupling of RAFT polymers to HEC, however the use of ^{15}N-labelled N_3-HEC and ^{15}N solid state NMR spectroscopy allowed unequivocal demonstration of the successful CuAAC reaction. Our strategy for preparing graft-copolymers of cellulose is efficient and produces extremely well-defined hybrid materials by combining RAFT polymerisation and CuAAC. Furthermore, we demonstrate that ^{15}N labelling and solid state ^{15}N NMR spectroscopy is an effective tool in probing CuAAC reactions involving poorly soluble polymer substrates.

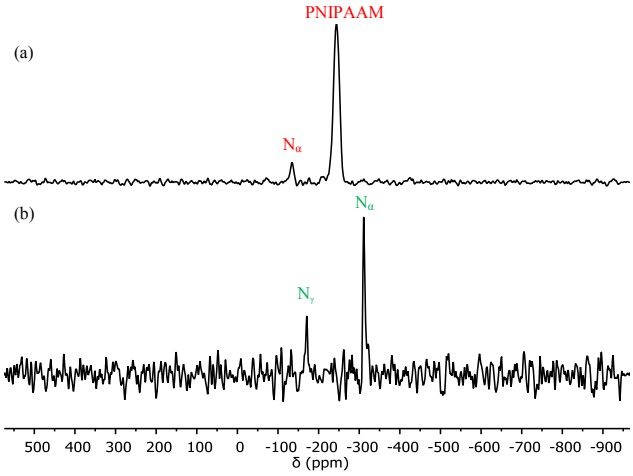


Fig. 8. Solid state ^{15}N NMR spectrum of (a) HEC-g-$PNIPAAM_{10}$ and (b) N_3-HEC.

Acknowledgements

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Notes and references

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Electronic Supplementary Information (ESI) available: schemes showing synthesis of CTAs, polymers and graft copolymers; characterisation of PVP and PNIPAAM; and FTIR spectrum of HEC-g-PNIPAAM. See DOI: 10.1039/b000000x/

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